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Review Paper

Application of microfluidic chip technology in pharmaceutical analysis: A review

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ABSTRACT

The development of pharmaceutical analytical methods represents one of the most significant aspects of drug development. Recent advances in microfabrication and microfluidics could provide new approaches for drug analysis, including drug screening, active testing and the study of metabolism. Microfluidic chip technologies, such as lab-on-a-chip technology, three-dimensional (3D) cell culture, organs-on-chip and droplet techniques, have all been developed rapidly. Microfluidic chips coupled with various kinds of detection techniques are suitable for the high-throughput screening, detection and mechanistic study of drugs. This review highlights the latest (2010–2018) microfluidic technology for drug analysis and discusses the potential future development in this field.

1. Introduction

Microfluidic technology has been developed rapidly in recent decades and offers multitudinous application in life sciences. The microfluidics revolution arose due to the distinct advantages offered by system miniaturization, including the high analytical throughput, enhanced sensitivity, improved analytical performance, facile parallelization through multiplexing, the ability to handle and process reduced reagent volumes and vastly reduced instrumental footprints.

Drug development involves several main processes, such as drug discovery, preclinical tests, and clinical trials, to develop a new drug for the market. Pharmaceutical analysis is prevalently utilized in all of these processes. However, this complicated process still faces some challenges from significant issues such as time consumption, low-throughput and cost. Microfluidics, as a miniaturization technology, could simultaneously provide analytical efficiency and high-throughput capabilities, without the loss of precision and automation. During the process of drug application, microfluidics technology is not only a powerful instrument for the rapid screening and analysis of drug discovery but also lower costs and reagent consumption by its miniaturized devices. The volume of the microfluidic chip device is minimal and many functions can be integrated on a chip of several centimeters. The internal dimensions of the chip range from micrometers to millimeters, so the consumption of the samples and reagents is at the nanoliter and picoliter levels [1,2]. Multichannel and array designs allow high-throughput to be achieved, which can increase the screening speed by hundreds of times and can decrease costs [3].

Since the pioneering development in the early 1990s, microfluidic technology has been applied in a variety of research fields including chemical synthesis [4], proteomics [5], single cell analysis [6], tissue engineering, high-throughput screening, environmental analysis and medical diagnostics. Such platforms provide new insights into biological processes and enable the efficient and rapid generation of novel pharmaceutical analysis. Microfluidic technology is being used to develop cost-effective in vitro models for lead compounds that can more reliably predict the efficacy, toxicity and pharmacokinetics of drug compounds in humans, as well as for novel screening assays. Microfluidic devices are recognized as advantageous not only for saving space and scarce/expensive materials but also because miniaturization offers several additional superiorities. This technology has become an increasingly promising tool for basic and applied research on drugs.

We introduce the application of microfluidic chips in drug analysis, including drug screening, drug determination, drug metabolism, toxicity, evaluation and other aspects. The innovations of these chips connected new research methods with drug analysis. Micro-droplets, 3D cell culture, organ microchips and other application are introduced coupled with recent literatures. This review provides a new insight into the research of drug analysis. Table 1 describes the significant use of microfluidic chips in this area and summarizes the device features and related representative literature [7–67].

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Table 1
Application of microfluidic chip in pharmaceutical analysis.

Type of microfluidic chip	Advantages	Disadvantages	Structural features	References
Droplet microfluidic platform	Precisely control the size of confinement as well as the density of cell seeding; Separate compartment; Very low consumption; Good repeatability; Rapid mixing; Faster response time	Complex manufacturing technology; Not often used for quantification; Limited detection parameters	Larger microbial population droplets	[7,8]
			In vitro microtumor models or cell encapsulation with 3D culture	[9,10]
			Enables on-demand trapping and releasing functions	[11]
			Droplet array technique	[12,13]
Organ-on-chip	Greatly reduced complexity of operation; Low consumption and cost; High throughput	Relatively single model; Difficult to fully exhibit the authentic functionalities of the organs	One-organ(skin/kidney)-on-a-chip	[14–18]
	Integration of multiple functional components; Establishing a complex model in one device; Investigateion of multiorgan interactions	Intricate design and manufacturing; Integrating all chambers on a single chip is difficult	Multiorgan chip	[19–22]
	Simulating complex disease models and external environments	Complexity of device fabrication and system setup	Co-culture of cells	[23–28]
Microfluidic chip combined with 3D culture technology	Mimicking the cellular microenvironment and recapitulateing the biological and physiological parameters of cells in vivo	Application range is not universal; Immature method for commercial promotion	3D cell culture technology	[29–35]
	Direct contrast highlights the advantages of 3D culture	Limited application	Compare the differences between 2D and 3D culture	[36,37]
Microfluidic hydrogel chip	Perform long-term cell culture; Hydrogel-based diffusion model similar to the natural tissue; Simplify equipment	Complicated operation process	Cells were encapsulated within alginate in microchannels	[38–43]
Microfluidic chip combined with detection instruments	Quantitative detection; Real-time measurement; Expanded application; Increased resolution	Some connections require special equipment; The detection limits are affected by the instrument itself; Existence of certain requirements for the instrument	CE	[44–46]
			UV	[47]
			Nano-HPLC-Chip-MS/MS	[48,49]
			Electrochemistry	[50–52]
			Chemiluminescence	[53,54]
			Biosensors	[55]
			Mid-IR	[56]
			Surface-enhanced Raman spectroscopy	[57–62]
			HPLC	[63]
Model organism	A holistic study of the embryo and its developmemt	Need to design a specific matching device	Experiments can be applied to whole-animals	[64,65]
Microfluidic chip for single cell research	Study cellular heterogeneity or subcellular structure of tumors; Single cell metabolism assay	Mostly used in scientific research, it is difficult to apply to clinical	Single-cell arrays	[66,67]

2. Drug screening

Drug development is a complicated process that involves several costly and time-consuming steps. For a compound to be developed into a new drug and reach the market, it requires an average of 10–15 years and costs from $1.5 billion to more than $1.8 billion. More robust and rapid methods to screen and validate potential drug candidates are urgently needed to provide efficient improvement and cost reduction for the drug discovery process. Compared to the conventional cell assay systems, the emerging microfluidic chip technology offers great advantages in high-throughput drug screening (HTDS) and has been accepted as an advantageous tool for cell biology research. In addition to the common advantages of microfluidics, including its high-throughput nature, low reagent consumption and potential for integration, the most influential benefit of microfluidics for cell-based drug-testing assays is the ability to reconstitute the cell microenvironment at the microscale [68].

High-throughput techniques are critically needed for the efficient screening of pharmaceutically valuable lead compounds. To identify effective compounds for a particular biological process, the rapid progress of high-throughput screening (HTS) enables parallel analyses of thousands of reactions. Most of the current HTS technologies involve robotics for automatic liquid and plate handling. Although the throughput has been increased tremendously by this automated technology compared to manual operations, the high costs associated with the instrumentation have restricted many researchers from performing HTS independently. As a result, there is a strong desire to develop low-cost technologies that require reduced sample and reagent consumption. Therefore, the advantages of the microfluidics-based high-throughput drug screening platform have gradually become prominent [69].

Over the past few years, significant progress has been achieved in developing microfluidics-based drug screening components and systems. Different forms of microfluidic chips are used for drug screening to increase the efficiency of screening and reduce costs. The following paragraphs list several common types of chip technologies.

2.1. Droplet microfluidics

Droplet microfluidic technology utilizes liquid droplets compartmentalized by an immiscible fluid as nanoliter to picoliter independent reaction vessels to carry out experiments in continuous or segmented flow. These approaches demonstrate considerable advantages such as reduced sample consumption, enhanced reaction speed and increased reliability and reproducibility, manipulating exceedingly small volumes with flexible control of composition.

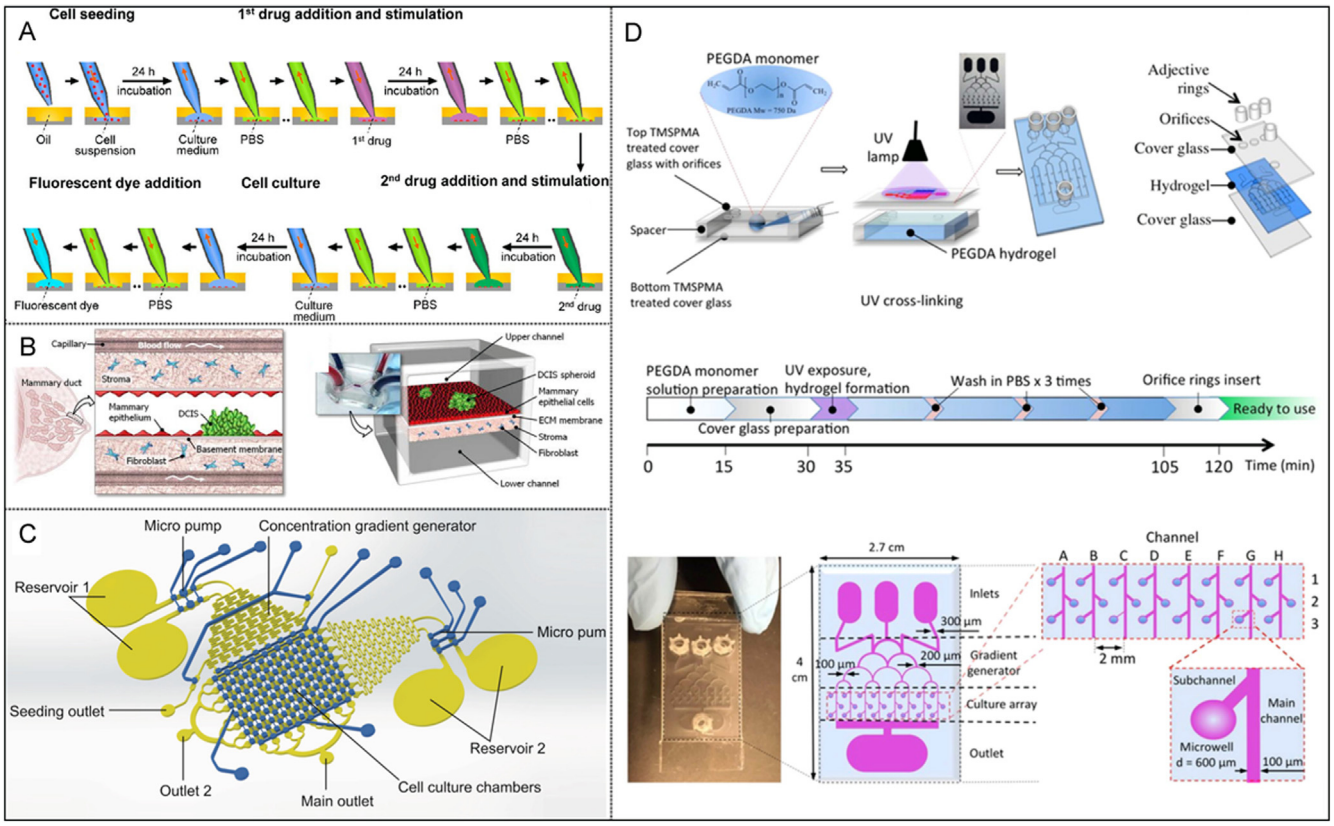

Fig. 1. Application of microfluidic chip in drug screening. (A) An illustration of a drug combination assay in the droplet array system in which the cells in the droplets were sequentially stimulated by two drugs [12]; (B) Human breast cancer-on-a-chip, left is a diagram of ductal carcinoma in situ(DCIS) embedded in a mammary duct and right is a microdevice reproduction of the microarchitecture of DCIS and the surrounding tissue layers [28]; (C) Different concentrations of sensitizer and drug are sequentially generated in the diffusive gradient mixers sequentially to perfuse cells cultured in downstream microchambers [77]; (D) Brain cancer chip design and preparation that contains a schematic of the layers, chip preparation time protocol, Christmas tree-shaped channel system and final hydrogel device with microchannels and microwells [88].

In a high-throughput manner, the droplet-based microfluidics have shown nanoliter to picoliter compartmentalization [7,8]. The applications of on-chip concentration gradients [70] and multicellular spheroids [9,10] expanded the application range of the microdroplets, simplified the screening process, made the established screening model closer to the actual tissue of the human body and enabled more reliable results. Compared to the traditional 96-well plate screening of drug compound libraries for potential bioactivity, droplet microfluidic platforms require much lower sample consumption (~200 times lower than a 96-well plate) and reduce reaction time due to the increased surface volume ratio (2.5 min vs 2 h) [11]. Droplet microfluidic methods can be used for drug combination screening based on the sequential operation droplet array technique (Fig. 1A), screen different dosing combinations and lengths of administration, and optimize the optimal dosing regimen with minimal consumption, which is meaningful for situations of combined diseases [12]. In addition, a low-cost slip chip provides an easy and economically viable method for performing microfluidic reactions without pumps or valves. Nevertheless, it should be noted that the commercial application of microdroplet chip technology is still rare and that the method is mainly applied to the use of specific equipment in a laboratory environment. Development of market-oriented production and operation is necessary.

2.2. Organ-on-chip

The precise control of structure and flow at the microscale permits the accurate modeling of organ tissue structures to be built at microscale. Organs-on-chips are micro-engineered biomimetic systems that represent important functional units of living human organs. To date, functions of various organs and tissues, such as the liver, kidney [19], lung and gut, have been reproduced as in vitro models. These systems can be used as in vitro models that permit simulation and pharmacological modulation of complex biological processes. Furthermore, a body-on-a-chip, which integrates multiorgan functions on a microfluidic device, has also been proposed for predicting organ interactions. Cancer is a serious worldwide human health problem with an incompletely elucidated pathogenesis; thus the study of cancer mechanisms and the discovery of low toxicity and effective drug from natural compounds are key to the development of anticancer drugs. Many organ chips are used in this field. This technology provides novel methods to mimic the in vivo tumor microenvironment in order to study drug effects.

The organ chip simulates the basic functions of the human body, meanwhile, it uses a variety of cells to construct a biomimetic chip with similar physiological functions on the special structure of the chip, which is closer to the real external environment of the disease than the traditional single cell culture model. It is an important breakthrough in chip applications that greatly improves the credibility of in vitro drug screening. In addition, multiorgan chips are connected according to physiological models, which can mimic the development of diseases in the system and can also enable the study of multiorgan diseases [20,21]. This has aroused great interest in the scientific community.

A microsystem that enables co-culture of breast tumor spheroids with neighboring cells in a compartmentalized 3D microfluidic device has been developed to replicate the complex microarchitecture of cancerous tissue, which is helpful for the

establishment of anti-breast cancer drug screening platform [28] (Fig. 1B). Co-culture of different tissues with a microfluidic system can be used to study cancer cell migration and anti-cancer drug screening [24]. A skin-on-a-chip mode microfluidic platform reduced the amount of culture medium and the number of cells required by 36-fold compared to conventional transwell cultures [14]. All of these platforms are simple to fabricate, handle and operate and can evaluate drug parameters, making them powerful in vitro tools for drug screening [15].

2.3. 3D cell culture

3D cell culture is a novel cell culture mode. With the unique structural characteristics of microfluidic chips, the advantages and applications of this culture mode are greatly improved [38]. The microfluidics device is a high-throughput method to screen drugs. However, most existing cell array technologies are based on two-dimensional cell cultures, which do not recapitulate the native in vivo microenvironment that is important for proper cellular growth, migration, differentiation, and patterning. In comparison, 3D tissue models offer the advantages of cell-cell, cell-extracellular matrix interactions [71], and spatial and physicochemical diversity which profoundly influence diseases. Additionally, they provide a sustainable, high-throughput 3D tissue formation platform, which can be used for drug screening [36,42,43] and enables us to define structure-function relationships and to model cellular study and disease progression [37,72,73]. In recent years, various microfluidic devices which enable the formation of cell spheroids in 3D have been applied to tumor spheroids in vitro to test anti-cancer drugs and to mimic the heterogeneous tumor tissue for cytotoxicity tests of anticancer drugs [40].

A microfluidic device can readily control the size of the tumor spheroids through the design of the cell culture chamber geometries in the device. Drug testing and analysis on a large number of uniformly sized tumor spheroids could be processed [29]. Microengineering methods to fabricate cell microarrays with 3D cell cultures that can be easily adopted by various labs to perform combinatorial assays can be used for drug-screening applications [30,31] and for long-term tumor spheroid cultivation and anticancer drug activity evaluation [32]. 3D cellular models mimic spatial cell-to-cell interactions present in vivo and have been proven to be accurate cell culture models. These models can replace animals in preclinical trials of drug candidates in certain aspects. 3D microfluidic devices are widely used for HTDS [33,34]. Some low-cost and easy-to-use chips have been introduced [38,39]. Microfluidic technology allows precise control over fluids in micrometer-sized channels and has become a valuable tool to mimic vessel vasculature. A new quantitative microfluidic angiogenesis screening microfluidic platform that can monitor and quantify cellular behavior in various concentrations of drugs has been applied [74]. An enormous potential for in vivo-like tissue-based application has been offered by the combination of 3D cell culture with microfluidic networks on a microchip [16,17,22].

2.4. Other microfluidic chips for drug screening

In addition to the abovementioned mainstream technologies, there are many other technologies applied to microfluidic chips for drug screening, which expands the researchers' ideas. An excellent HTDS system has become the most important and integrated aspect of drug discovery in most pharmaceutical and many biotechnology industries worldwide. Researchers utilize a large number of experimental platforms to screen a variety of concentrations/combinations to identify an appropriate sensitizer and to find effective drug combinations for disease treatment.

It is possible to screen different concentrations and combinations of drugs through the application of open-access microfluidic tissue array systems [75] and cell microarray chips [76]. The different designs of the chip can generate various combinations and arrays of small culture chambers. The consumption of cells and reagents is greatly reduced; the processing is performed in a flexible and simple way and the function of automatically screening complex combinations is realized with further improved devices. Concentration gradient generators can produce an accurate gradient of liquid concentration, and some research groups have applied and combined these devices with microfluidic technologies to optimize HTDS systems [70,76]. The microfluidic diffusive mixers in Fig. 1C can also realize a fully automated HTDS: each diffusive mixer contains two integrated micropumps connected to the media and the drug reservoirs without the need for any extra equipment to perfuse the solution; this device minimizes drug consumption and does not require the continuous generation of solutions [77]. In addition to the consideration of the high-throughput factor, cost and various consumptions are also our focus. Traditional drug screening efforts rely on the use of 96-well plates to assess the efficacy of various drug candidates against key biological targets [78]. In this screening method, manual pipetting or robotic handlers were used to dispense reagents into wells, and these platforms consumed large amounts of expensive biological components and reagents [79]. A 96-well-formatted microfluidic plate with built-in micro-gaps used small amounts of cells for a single reagent test, enabling drug screening and compatibility with conventional automated workstations. This device enables precious primary tumor samples to be subjected to high throughput screening of cancer drugs [80].

In some microfluidic channels for drug screening, cell array platforms are constructed using material made from polydimethylsiloxane (PDMS) [81]. The drugs flow through the microfluidic channels to compartmentalized cultured cells [82,83]. This chip material is widely used because of its many advantages such as optical performance and durability, and biocompatibility. However, the structure of the chips is complicated and confers some limitations, such as the expensive silicon molds and the absorption of biomolecules. Poly (ethylene glycol) diacrylate (PEGDA) hydrogel possesses similar mechanical properties and water content as natural extracellular matrix (ECM). This material is photopolymerizable, so it can be easily and quickly synthesized. PEGDA microfluidic hydrogels are permeable to substances such as water, biomolecules, and chemicals [84,85] and have been widely used for cell encapsulation. In addition, they can also entrap and release drugs through diffusion [86,87]. Microfluidic devices composed of these kinds of materials and coupled with 3D brain cell culture technology were used to study the combinatorial treatment effect of two drugs (Fig. 1D) [88]. A hydrogel-based tissue-mimicking structure with microfluidic channels for simulating the drug diffusion model was developed for drug screening and cell viability assays, offering the features of real-time monitoring capability combined with the advantages of non-invasion, label-free detection, time saving and simple manipulation [41].

The allure of the microfluidic chip is that it can include different designed structures to achieve varied functions and can be combined with different devices and testing equipment to expand its application range. However, it requires significant efforts to design, manufacture and optimize. Each design has its own particular characteristics. It is not only used for drug screening but also for drug testing.

3. Drug detection

Numerous methods based on liquid chromatography [89], spectrophotometry, immunoassay, electrochemistry [90], gas chromatography/mass spectrometry [91] and electrophoresis [92] have been developed for drug testing. These detection methods

are re-glowed after the combination of microfluidic chips. Enzyme-linked immunosorbent assay (ELISA) [93,94], electrochemical biosensors [95] and point-of-collection testing devices [96] are relatively new methods. However, the immunoassay method requires specific antibodies and relatively long analytical time. The narrow linear ranges of electrochemical biosensors limit their practical application [97]. Microfluidic detection technology is recognized as one of the most promising analytical tools due to its admirable merits, such as its small footprint and energy consumption, minimal use of reagent, and waste production, in addition to the widely known and relatively easy fabrication methods. Real-time analysis has also been described in microfluidics [45,46]. Therefore, it is especially suitable for therapeutic drug monitoring (TDM) [98] and plays an important part in an emerging technology of point-of-care testing [99]. This is a quick, cost-effective method for detection of low-level drugs such as those in biological or other complex fluids that would be of great value in healthcare, law enforcement, and home testing application. It greatly improved the quantitative detection capability of microfluidic technology and expanded the application area.

A renewable hybrid microfluidic device with UV detection could identify and quantify three kinds of psychotropic drugs [47]. This protocol, integrating the extraction and concentration procedures on a single chip, could further reduce the analytic time and increase the detection sensitivity. The baseline separation of these drugs was achieved within 200 s with a separation efficiency up to 3.80×10^5 plates/m. This is a major improvement. A microfluidic chip based nano-HPLC coupled to tandem mass spectrometry (nano-HPLC-chip-MS/MS) has been developed for the simultaneous measurement of abused drugs and metabolites [48,49]. There are just a few examples of new applications for conventional detection methods. There are some applications in

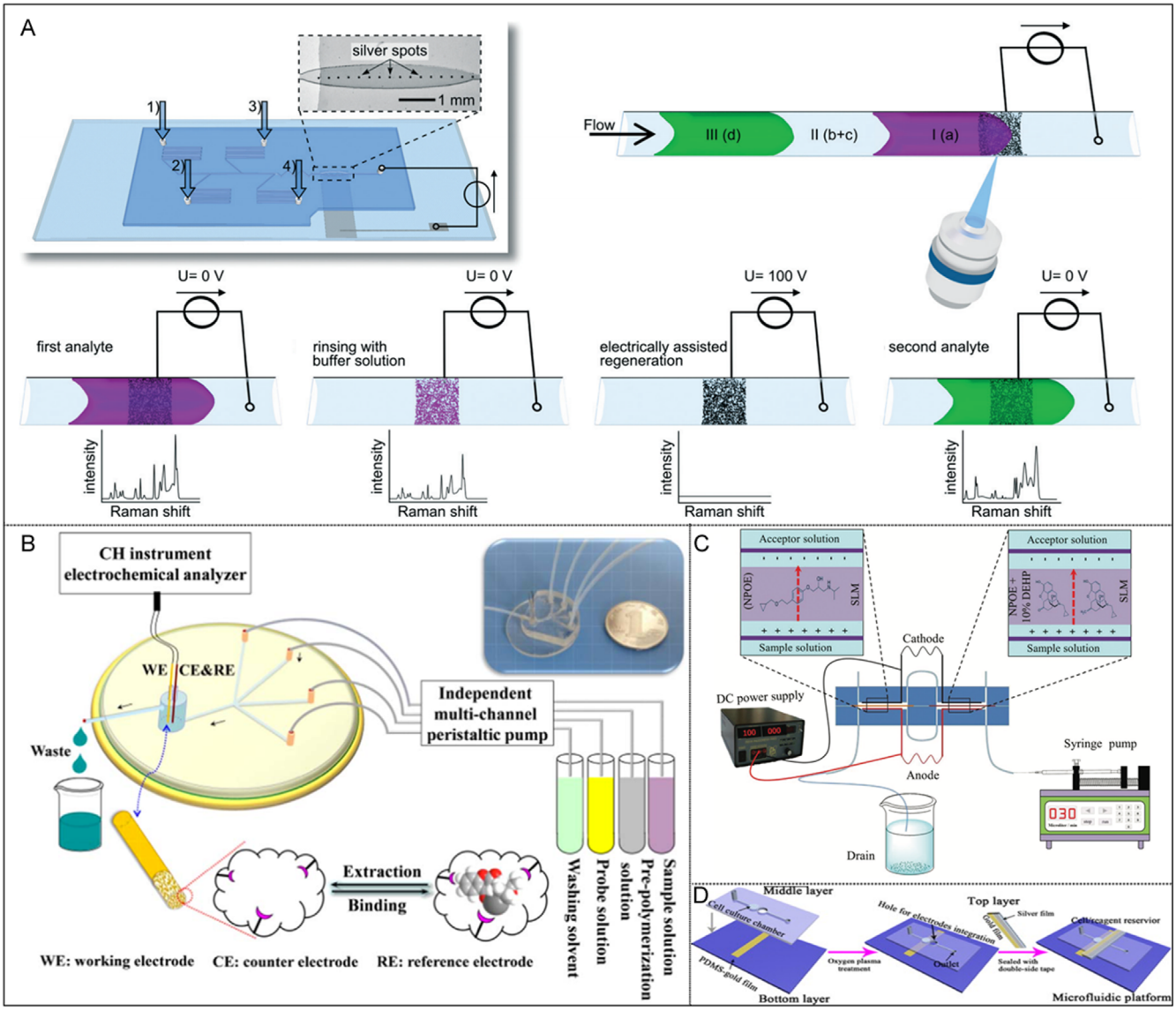

Fig. 2. Application of microfluidic chip in drug detection. (A) Schematic drawing of the experimental setup and the process for electrochemically assisted regeneration of integrated SERS substrates in a microfluidic chip. The chip with four inlets (1–flushing buffer, 2–malachite green, 3–brilliant green and 4–crystal violet) and an outlet channel, as well as the actual regeneration process with two different analyses, are schematically sketched [59]; (B) Schematic illustration of chip structure of an actual photograph and its application in detecting WFS. The chip has four inlets connected with an independent multichannel peristaltic pump; these inlets were respectively used for injecting four kinds of working or sample solutions into the detection chamber [52]; (C) Schematic illustration of consecutive on chip EME procedure. The chip consists of two PMMA parts into which two microfluidic channels are carved in each part, these channels were used as a flow path for the sample solution and a thin compartment for the acceptor phase [104]; (D) Construction process of the sandwich-type microfluidic electrochemical cytosensing platform; the device was constructed from three layers of PDMS slab with a gold film working electrode (bottom layer), flow channel, cell culture chamber (middle layer), gold film counter electrode, and silver ink reference electrode (top layer) [50].

TDM based on electrochemistry in pharmaceutical analysis [50,51]. A flexible PDMS-based electrochemical cytosensor device can perform real-time monitoring (Fig. 2D). Chemiluminescence detection is more readily combined with the chip and was also used for drug study [53,54]. A novel electrochemical detection platform was established by integrating a molecular imprinting technique with microfluidic chips and was applied for trace measurement of three therapeutic drugs [52]. The system was then applied for 24 h monitoring of drug concentration in plasma and the obtained corresponding pharmacokinetic parameters (Fig. 2B). Chips integrated with biosensors are also widely used in forensics. A chip implemented real-time drug testing and the rapid sensing of small molecules using biosensors composed of biological nanopores conjugated with DNA aptamers [55] These microchips detected a low concentration of cocaine (300 ng/mL), with the drug test cutoff limit) within 60 s. A chemical sensor utilizing a mid-IR single-mode strip waveguide integrated with a microfluidic chip was used to detect cocaine [56]. A review of microfluidic platforms used for forensic drug analysis summarized that these new technologies offered great opportunities for on-site drug testing [100]. In addition to these detection methods, additional new applications offer good prospects.

Within the past ten years surface-enhanced Raman spectroscopy (SERS) has become increasingly important within the field of microfluidics due to its potential for label-free, nondestructive and sensitive on-chip detection, suitable for the detection of trace drugs and for the monitoring of drugs. A device controls and optimizes the interactions of microliter samples of saliva with a SERS substrate based on silver nanoparticles (Ag-NPs) in suspension, enabling the rapid detection of a drug at biologically relevant concentrations [57]. Kline et al. [58] detected morphine, cocaine, and methamphetamine with the lowest detection limits, and this study was carried out to determine the optimal SERS conditions for detecting drugs using Au and Ag nanoparticles as a SERS substrate for implementation in a microfluidic device. Other kinds of materials were used as medium, such as colloidal nanoparticles [101,102]. Multiple groups have also designed devices that utilize immobilized SERS substrates such as TiO_2 nanotubes and noble metal films over nanostructured black silicon [59,60] (Fig. 2A). SERS was also used to detect sulfamethoxazole in drinking and surface water sources [61]. A review summarized SERS-based detection platforms [62].

Paper chips can be used for rapid detection because of their ease of processing and low cost [66]. Koesdjojo et al. [103] presented the production of a rapid, inexpensive and simple colorimetric-based paper microfluidics testing kit for the detection of counterfeit artesunate in order to preserve life and prevent the development of multidrug resistant malaria. The assay costs approximately $0.02 per test, and the result was obtained within minutes. Electromembrane extraction device chips may also be good alternatives for the extraction and determination of drugs [104] (Fig. 2C) and for monitoring drug metabolism in real time [105]. An on-chip liquid-phase micro-extraction coupled with high-performance liquid chromatography for the extraction and analysis of some hormonal drugs has also been reported [63].

4. Application of microfluidic chips in drug metabolism, evaluation of drug toxicity and other aspects

4.1. Drug metabolism

A drug candidate experiences a complex in vivo ADME process (absorption, metabolism, distribution and excretion). Traditional in vitro drug screening tools cannot effectively recreate the ADME process of a drug candidate in the body and, consequently, frequently yield biased screening results and may ultimately lead to failure during the animal testing or clinical trial stage. Pharmacokinetics of a new drug plays an important role in the evaluation of therapeutic effect and toxicity. The existing experimental methods require laborious human work on the mixtures of drugs and the manipulations of cells [7]. Furthermore, those detection techniques cannot provide real-time fingerprinting of intracellular drugs. Microfluidic chips may allow the co-culture of multiple types of functional cells and a comprehensive analysis of cells, drug candidates and their metabolites [25] (Fig. 3A) in a simple and automated manner.

Bioartificial organs cultivated in microfluidic culture conditions provide a beneficial environment in which the cellular cytoprotective mechanisms are enhanced, compared with Petri dish culture conditions [106]. The study can provide an important insight into the use of microfluidic biochips as new tools in pharmaceutical drug studies and predictive toxicity investigations. This method can illustrate the potential of metabolomics-on-a-chip as an in vitro alternative method. This kind of chip enabled drug assessment regarding the production of basal liver biomarkers and the performance of drug metabolism [107]. The metabolic clearance of seven drugs was also investigated by microfluidic biochips [108]. The co-culture of multiple types of functional cells in microfluidic chips could be seen as a new variation of ADME screening platforms and could enable the comprehensive analysis of cells, drug candidates and their metabolites simultaneously. This technology has been widely used [23,26,27].

In addition to the use of common disease cells, different animal models were used to analyze drug metabolism and the drug effectiveness towards a target model. *Caenorhabditis elegans* (*C. elegans*) represents a powerful model for such aging and metabolic studies because of its relatively short lifespan of 2–3 weeks. Researchers could study the effects of drugs on the development of *C. elegans* and characterize the metabolism of drugs [64]. Similarly, the zebrafish model-integrated microfluidic device was also used to monitor dynamic drug effects for embryonic research [65].

Optical detection is a quick and simple method for drug metabolism research, but it is difficult to obtain quantitative and accurate measurements. Some analytical instruments can make up for this shortcoming. A screening method using an electrochemical chip coupled online to MS or LC-MS was utilized to generate phase I and phase II drug metabolites and to demonstrate protein modification by reactive metabolites (Fig. 3C). This method allowed researchers to detect short-lived metabolites [109]. Regarding electrochemistry, a powerful analytical technique for chemical analysis of living cells, biologically active molecules and metabolites, Gao et al. [110] reviewed useful measurement tools such as the electrochemical biosensor, microfluidics and mass spectrometry for various fields of biology and medicine. Similarly, to the above drug detection, the same cell culture mode and detection method can be used for the determinations of pharmacokinetic parameters. For example, a programmable microfluidic platform using cell co-culture techniques monitored intracellular SERS spectra to perform pharmacokinetic detection [111]. A biosensor was integrated into an automated microfluidics platform for investigating the mechanism of action of a hypoglycemia drug [112]. Many studies on drug metabolism have been combined with other aspects of research, such as the investigation of cell morphology, and the determination of drug structure or drug concentration, all of which explain the role of drugs in the model.

4.2. Evaluation of drug toxicity

The evaluation of drug toxicity is essential for the safe development of novel pharmaceuticals and is generally based on animal and cell-based models; however, the in vivo tests present drawbacks including ethical issues, high costs and the impossibility of

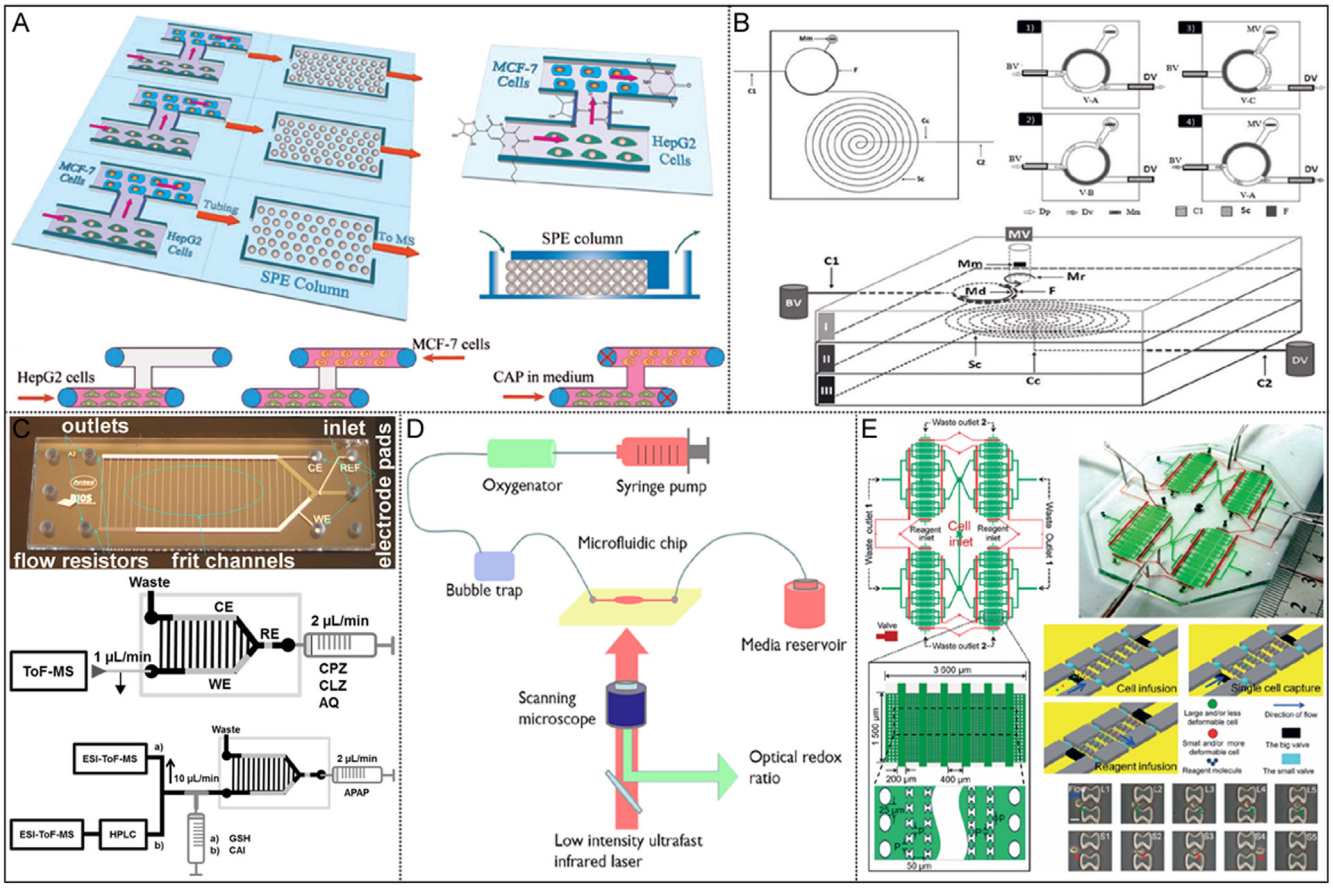

Fig. 3. Application of microfluidic chip in other aspects of drug analysis. (A) Schematic of the microfluidic device and cell co-culture process, microchip with parallel units for cell co-culture, drug metabolism and metabolite extraction and specific details [25]; (B) Schematic diagrams showing the layout of the fabricated microfluidic chip-CE device and the sequential four-step operation of sample loading for the device [44]; (C) Miniaturized electrochemical cell, schematic diagram of the EC/ESI-MS setup used in phase I & II metabolism studies and protein modification studies [109]; (D) Schematics of the microfluidic system. It is composed of a culture medium reservoir, a syringe pump, an oxygenator, a bubble trap, and a chip and was imaged on a two-photon imaging system [114]; (E) Integrated microfluidic system for single-cell separation according to cell size and deformability, which combined multiobstacle architecture-like microstructural matrices and a microvalve system. Using this device, the biomechanical (size or deformability) heterogeneity of normal and induced glioblastoma cells was studied on a single-cell level [67].

performing quantitative studies or high throughput assays, which limit their use. The rapid development of organ-on-chip technology may provide a good solution to simulate the complex in vivo physical environment in vitro [113].

The monitoring of cells and tissues is an important aspect of judging drug toxicity, and monitoring of various indicators simply, efficiently and accurately is the direction towards which many researchers are striving. A noninvasive imaging-based assay on microfluidic chips was utilized to evaluate drug toxicity response (Fig. 3D). The simultaneous determination of two parameters was used to monitor changes in cellular metabolic activity as a result of drug treatment [114]. In recent years, organ chips were also used for drug toxicity studies. An intestine-kidney-on-a-chip with multi-interfaces and compartmentalized micro-chambers could effectively assess drug absorption-related nephrotoxicity [19]. Similarly, a kidney-on-a-chip capable of simulating the nephron was used to investigate the pathophysiology of drug-induced acute kidney injury and to provide assessments of drug-induced nephrotoxicity [18]. The toxicity of drug compounds can also be investigated by microfluidic chips. For instance, Su et al. [115] utilized a high-throughput, reliable, and less costly platform to study the growth and culturing properties of HEK cells, aiming towards screening cardiac toxicity.

Recent advances in microfluidic cell cultures enable the construction of in vitro human skin models that can be used for drug toxicity testing and disease study. A 'skin-on-a-chip' approach was developed to mimic the structures and functional responses of the human skin to evaluate the effects of therapeutic drug testing models [15]. Hepatotoxicity is also an important aspect of people's concern. Ma et al. [116] have developed a microfluidics-based biomimetic approach for the fabrication of an in vitro 3D liver lobule-like microtissue. They successfully analyzed the potential adverse drug reactions that induced liver injury via drug–drug interactions of clinical pharmaceuticals. A related review summarized and discussed the microfluidics-based in vitro systems realized to study liver metabolism and toxicity with respect to their applications, advantages, and limitations [117].

4.3. Drug evaluation

The drug evaluation is also an important aspect of drug analysis. Study of drug-cell interactions can provide valuable drug evaluation information. Meanwhile, drug safety and resistance are additional indispensable components.

According to different diseases, the unique microfluidic platform is designed to simulate a similar tissue structure. After administering different drugs, related indicators are monitored to evaluate the effectiveness, toxicity and safety of the drug. The blood-brain barrier (BBB) is an important factor affecting the use of drugs in the brain. Wang et al. [118] developed a sophisticated microfluidic BBB model that is capable of mimicking in vivo BBB characteristics for a prolonged period and allows for reliable in vitro drug permeability studies under recirculating perfusion. The

application of microdroplets which could encapsulate different types of gastric cancer cells together enabled the study of the transfer pathways in the model and the expression of transfer proteins, and explained the mechanism of chemical sensitivity [10]. An efficient droplet microfluidics-based approach assessed the dynamics of drug uptake, efflux and cytotoxicity in drug-sensitive and drug-resistant breast cancer cells [13]. Pang et al. [67] established an integrated microfluidic platform for the construction of single-cell arrays and the analysis of drug resistance. Fig. 3E shows the design and principle of the device. The effect of the biomechanical heterogeneity of cells on their biological characteristics was studied. The results indicated that the biomechanics of single glioblastoma cells had significant implications for cellular drug resistance. A 3D tumor model in a microchannel chip was an excellent apparatus to study cell migration, cellular transition, and drug resistance as well as the underlying molecular mechanisms [35,36]. Hypoxia effects in tumor cells could be studied by a microfluidic culture system capable of rapid switching of local oxygen concentrations, and the results show that exposure to oxygen for a similar time can reverse changes in the cancer cell drug resistance [119].

The content of drug evaluation is very rich and is closely related to the content described above. Herein, only a few examples are listed. The different designs have different foci. It is worth learning from all of these advancements.

4.4. Chiral drug separation

Enantiomers of a racemic drug often differ considerably in pharmacological activity, pharmacokinetics, and toxicity. Chiral separation has always been a research challenge and must be solved urgently. Therefore, it is essential to develop suitable analytical methods for the separation of enantiomers. Microfluidic chips offer advantages in this area. With the aim of developing an easily operable and low-cost device for bedside emergency monitoring of potent drugs, a microchip-CE device with adjustable on-chip dilution and separation functional units was developed for enantioseparation of blockers in human urine [44]. A spiral design could be observed. An article reviewed the most recent advances in the use of cyclodextrins as chiral selectors in capillary electrophoresis for the enantioseparation of drugs [120]. This publication primarily summarizes the applications of microfluidic chips in drug metabolism, drug toxicity and drug evaluation. The development and application of microfluidic technology provides more choices and options for increased understanding of the role of drugs.

5. Conclusions

Pharmaceutical analysis is an important area of drug research involving all aspects within the endeavor. At present, traditional drug analysis methods, including routine separation, detection, drug metabolism, and the mechanistic study of drug interactions, have limitations when performed at a macroscopic level. The most common are the complex and time-consuming sample preparation and the low-throughput nature of drug detection. More critically, it is difficult to evaluate multiple aspects of the drug at the same time. The development of microfluidic devices and the application of microfluidic chips have enabled entirely new research methods for drug analysis. The study of microfluidic flow occurs at the micro or nano level, and this characteristic can provide new perspectives for drug research. Microelectromechanical processing technology and the joint application of detection technology are improved rapidly in a short amount of time. These chips can be made into extremely complex microchannels and domains and can be combined with substrates and additional chips of different materials to achieve different functions, such as liquid flow, cell culture, and concentration gradients, through various control valves. One can also study multiple properties regarding drugs and cells by applying different detection methods.

Microfluidic chips are now used in the vast majority of aspects of drug research, including drug screening, development, testing, toxicity, sensitivity, drug resistance assessment, drug metabolism, pharmacokinetics, the chiral separation of drugs and drug interactions. Lab-on-a-chip-based in vitro and in vivo models, which are mainly used for phenotypic screenings in the preclinical phase, allow for higher reliability and better control than traditional in vitro assays. The application of 3D cell culture and body-on-chip chips can better mimic the actual body environment, which is an important advancement. The improved cell culture techniques will have a vital role in all future cell-based assays and will increasingly contribute to the development of novel drug discovery tools in combination with well-established methods. Researchers developed the organ-on-a-chip technology with the combination of microfabrication technology and bioengineering technology. This technology and droplet microfluidics enable reduced consumption of cells and reagents, which indicates enhanced potential for industrial high-throughput application. The new detection technology and unique design allow researchers to easily detect trace amounts of drugs in biological samples, as well as to detect metabolic processes and metabolites of drugs. Compared with traditional whole animal experiments and in vitro testing methods, overall, microfluidic chips confer obvious advantages. However, the complex design of microfluidic chips, the stable attachment and integration of different functional organ chips, and the modification and development of different culture conditions in a variety of cell co-cultures require further optimization and experimentation. The problems of molecular absorption, mass transfer, and bubble generation inside the equipment necessitate urgent solutions. Good operational stability and repeatability are essential for the promotion of the method. In addition, the high cost and complicated preparation and operation processes are also issues requiring attention. If more convenient, user-friendly and inexpensive devices can be developed, the microfluidic chip promises to play a greater role in pharmaceutical analysis.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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